dNTP" reactions Y639F was least active in the dGTP, dATP reaction, corresponding to the 2 nucleotides incorporated first during transcription. In the "3 dNTP" reactions Y639F was least active in the dGTP, dATP, dCTP reaction, corresponding to the 3 nucleotides incorporated first during transcription.

In Mn^{++} buffer both the w.t. enzyme and Y639F show a reduction in their sensitivity to substitution of dNTPs for rNTPs, consistent with an expectation of reduced substrate discrimination in Mn^{++} buffer. There was, however, also a sharp reduction in overall activity with Mn^{++}. We varied Mn^{++} concentrations over a wide range (from 20 mM to 150 μM in 2-fold dilutions) to determine if an optimal Mn^{++} concentration that would result in high activity could be identified, but we found similar activity at all Mn^{++} concentrations tested (data not shown). Thus, while discrimination between rNTPs and dNTPs was less in Mn^{++} buffer, Y639F is more active in Mg^{++} buffer than in Mn^{++} buffer with all NTP combinations examined. Similarly, the w.t. enzyme exhibits greatly reduced discrimination between rNTPs and dNTPs in Mn^{++} buffer, but is modestly more active in Mn^{++} buffer than in Mg^{++} buffer only for certain combinations of dNTPs and rNTPs.

DNA and RNA synthesis on homopolymeric templates: T7 RNAP will synthesize poly(rG) RNAs on poly(dC) templates (Bonner, et al., 1994; Ikeda and Richardson 1987). We measured the activity of the w.t. and mutant polymerases on poly(dI)•poly(dC) with rGTP, dGTP, dGTP+rGMP (Table III, see Appendix 1). The activity of T7 RNAP on poly(dI)•poly(dC) and poly(dC) is especially robust. Mutant polymerases that have greatly reduced activity on normal promoter templates still display high activity on poly(dC) templates (Bonner,

et al., 1994). We, therefore, characterized two poorly active non-conservative tyrosine mutations on this template (Y639A and Y639S). In Table III we also present results obtained with mutant G640A. Presentation of data for the latter mutant was selected because it is more comparable in activity to the Y639A/S mutants and because it is representative of a mutation which has marked effects on the kinetics of transcription but does not affect substrate discrimination, even though it is directly adjacent to Y639. We find that all of the Y639 mutants exhibit reduced substrate discrimination as demonstrated by the fact that their differential activity in reactions containing dGTP or dGTP+rGMP vs. rGTP is less than for the w.t. enzyme or the G640A mutant. In fact Y639F displays similar activity with rGTP, dGTP, or dGTP+rGMP.

Since the nucleic acid synthesized by Y639F on poly(dI)•poly(dC) using dGTP is presumably composed solely of dNMPs it is expected to be resistant to alkaline hydrolysis (Schmidt and Tannhauser, 1945). Fig. 4 shows transcription by Y639F and w.t. polymerase with dGTP or rGTP on poly(dI)•poly dC). Transcription reactions were carried out with poly(dI)•poly(dC) at .2 mg/ml and the indicated NTPs and polymerases. Reaction products were left untreated (-) or treated with 1 M NaOH for 5 hours at 37°C (+). Polymerase and NTP concentrations and electrophoresis as in Fig. 1. Labeling was with α-P^{32} rGTP (a, d, g, j) or α-P^{32} dGTP (other lanes). Fig. 4 shows the transcription products obtained with the w.t. or Y639F polymerases on poly(dI)•poly(dC) before and after treatment with alkali. With dGTP or dGTP+rGMP the w.t. enzyme is poorly active in the synthesis of long transcripts, however we can observe smears of heterogeneously sized, short transcripts in the

reactions with the w.t. enzyme and the dGTP substrates (lanes e and f) while Y639F synthesizes higher levels of long transcripts which are retained near the top of these gels (lanes b and c). The presence of these short transcripts indicates that Y639F and the w.t. enzyme differ only in the degree to which they can utilize dNTPs. The w.t. can also initiate and extend transcripts with dGTP, but it is much less processive when using dNTPs than Y639F so its transcripts are much shorter. When these reactions are treated with base, degradation of the long transcripts made in the reactions with rGTP is observed and the amounts of short RNAs (presumably hydrolysis products) increase (lanes g and j). In the reactions in which dGTP or dGTP+rGMP were used as substrates no degradation of the transcripts by base treatment is observed, confirming that these transcripts are composed of dNMPs.

T7 RNAP as a reverse transcriptase or RNA replicase which initiates *de novo*: It has been reported that T7 RNAP can use both RNA and DNA templates (Konarska and Sharp, 1989). We, therefore, determined if the Y639F mutant would use dNTPs when transcribing an RNA template (poly(rC), Table IV). Overall the activity of the w.t. and Y639F polymerases on poly(rC) with rGTP was 10-20-fold less than on poly(dC) (not shown), but this reduction did not preclude synthesis of high levels of RNA on poly(rC) by using higher polymerase concentrations than were used in the poly(dI)•poly(dC) reactions. When dGTP or dGTP+rGMP was used the w.t. enzyme was not measurably active on poly(rC), while the activity of Y639F was reduced by only ~4-fold (with dGTP+rGMP) or ~8-fold (with dGTP). Thus, both the w.t. and Y639F polymerases are capable of unprimed RNA-directed RNA polymerization